Journal on New Biological Reports
JNBR 4(2) 149 – 156 (2015)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Efficacy of a potential lichen *Parmotrema andinum* (Müll. Arg.) Hale against pathogenic microorganisms

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| **Received**: 16 May 2015 | **Accepted**: 20 June 2015 |

ABSTRACT

The present study was conducted to evaluate the *In-vitro* antimicrobial activity of 2-Propanol, acetone, methanol, petroleum ether and aqueous extracts of *Parmotrema andinum* (Müll. Arg.) Hale against each eleven human pathogenic bacteria and fungal pathogens. Secondary compounds of the species were extracted with Soxhlet apparatus and antimicrobial activity was carried out by using Kirby-Bauer disc diffusion method. The extracts were found more effective against all bacterial and fungal pathogens. Among the eleven bacterial pathogens highest zone of inhibition with 2-propanol was recorded against *Corynebacterium rubrum* (10.3±0.5), *Salmonella typhimurium* (10.0±2.6) and *Pseudomonas aeruginosa* (9.6±1.5) followed by lowest inhibition zone recorded in *Enterobacter cloacae* (7.3±0.5) and *Salmonella abony* (7.3±0.5). The highest and lowest zones of inhibitions with acetone extract were reported against *Enterobacter cloacae* (8.3±2.3) and *Salmonella abony* (4.6±4.0). The highest and lowest zones of inhibitions with petroleum ether were recorded against *Salmonella abony* (9.3±2.1). Streptomycin was taken as standard control found more effective against all the bacterial pathogens. In case of fungal pathogens the highest zones of inhibition with 2-propanol were noted against *Trichoderma lignorum* (22.66 ±6.17) followed by *Aspergillus niger* (21.33±1.20) and *Fusarium moniliforme* (20.66±0.88). The highest zone of inhibition with acetone extract was recorded against *Rhizoctonia bataticola* (14.33±1.20) and lowest zone of inhibition recorded against *Fusarium oxysporum* (5.33±2.72) while commercially available synthetic antifungal drug Ketoconazole was taken as standard control found more effective against all fungal pathogens. The study revealed that extracts obtained from *P. andinum* having potential compounds which in turn are useful to control the diseases caused by these pathogens against human beings and plants.

Key Words: Macrolichen, antimicrobial assay, Kirby-Bauer disc diffusion method, bioactive compounds.

INTRODUCTION

Lichen is an association of a fungus and a photosynthetic symbiont resulting in a stable thallus of specific structure Alexopoulos and Mims (1979). The thallus of lichen is unique in nature and morphology because of its appearance and behaves quite differently from its component organisms.

Lichens produce a wide range of organic compounds that can be grouped as primary metabolites and secondary metabolites Elix (1996). Lichens and their secondary compounds have been used in traditional medicine for centuries and even today they hold considerable interest as an alternative medicine in various parts of the world Richardson (1991). The secondary metabolites of lichens are unique with respect to those of higher plants Hale (1983) & Lawrey (1986). The presence of antibiotics in lichens was first reported by Burkholder *et al*. (1944). According to one appraisal, 50% of all lichens have antibiotic properties Sharnoff (1997). The secondary metabolites of lichens *Cetraria islandica*, *Lobaria pulmonaria* and *Cladonia* sp. were known for the treatments of Pulmonary Tuberculosis Vartia (1973). Lichen metabolites were utilized for a wide variety of biological actions including antibiotic, anti-mycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects Muller (2001).

A total 137 lichen species have medicinal properties and their occurrence in India is enumerated, of which 36 species were used in traditional medicine in India or in the world, 55 species have been screened for antimicrobial activity, about 57 species for antioxidant activity and 37 species were used in treatments for anti-cancer and cytotoxicity Nayaka *et al.* (2010). The present study reports on the extraction of secondary metabolites of *P. andinum* using organic solvents and antimicrobial activity exhibited by the different solvents of the crude extracts against the human bacterial and fungal microbes.

P. andinum (Müll. Arg.) Hale, is common Parmelioid lichen found growing luxuriantly over the surface of rock. It is characterized by greenish to pale grey with white maculae, lacking isidia, soredia and cilia, lower side of the thallus centrally black with wide nude marginal brown zone, medulla white, secondary compounds consist of lecanoric and orsellinic acids. The species also has a cosmopolitan distribution in the world Divakar (2005) & Awasthi (2007) and found to be very useful to the mankind. The lichen species *P. andinum* (=*Parmelia paraguariensis*) is used in Mauritania as tobacco Hawksworth (2003). Since there were no much imperial booms on *P. andinum* the current study has been focussed for the evaluation of antimicrobial activities

MATERIALS AND METHODS

Collection and identification of sample

P. andinum thalli (Fig. 1A) were collected from Sundupalli forest area, YSR District, Andhra Pradesh at an altitude of ca. 500–600 m. The specimen identification was carried out by following the standard procedures given by Nayaka (2014). The literature of Awasthi (2007) was referred for taxonomic characters and Orange *et al*. (2001) was followed for chemical analysis. The voucher specimens were deposited at the Department of Botany, Yogi Vemana University, Herbarium (YVUH), Kadapa, Andhra Pradesh, India.

Extraction of bioactive compounds

Lichen thalli were washed using distilled water and dried at room temperature for 24 hrs to remove the moisture contents completely. Subsequently it was powdered using a pestle and mortar. Around 10 g of powdered lichen samples was wrapped in Whatman No.1 filter paper which was kept inside the extractor tube of Soxhlet apparatus. 150 ml of 2-Propanol, acetone, methanol, petroleum ether and water was used in Soxhlet apparatus for the extraction of bioactive secondary metabolites from lichen thallus. Few drops of the extract were used for Thin Layer Chromatography (TLC) to identify the major secondary compounds. The obtained extract was concentrated in vacuo at 40°C using a Heidolph rota vapour. The obtained residues were preserved in a Deep Freezer at -80°C until they are used for future study.

Microorganisms and media

The microbes used in the present study were procured form NCIM (Pune). Eleven bacterial pathogens viz. *Bacillus cereus, B. subtilis, Corynebacterium rubrum, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella abony, S. typhimurium, Staphylococcus aureus, Streptococcus pyogenes* were used and maintained on Muller Hington Agar (MHA) media at 37°C and fungal pathogens *viz. Aspergillus niger, A. flavus, Colletotrichum falcatum, Fusarium moniliforme, F. oxysporum, F. solani, Mucor* sp. *Penicillium chrysogenum, P. notatum, Rhizoctonia bataticola* and *Trichoderma lignorum* were used and maintained on Potato Dextrose Agar (PDA) at 27°C.

Screening of antimicrobial activity

The antimicrobial activity of crude lichen extracts was studied by Kirby- Bauer disc diffusion method using pathogenic bacterial and fungal micro-organisms Bauer *et al*. (1966). Bacterial cultures (1.5×10^8 CFU/ml) were seeded onto the Muller-Hinton Agar plates with the help of L – spreader. The fungal mat of 2–3 days old cultures grown on Potato Dextrose Broth were collected. These cultures were inoculated onto PDA plates for fungal pathogens and MHA for bacterial pathogens. On the seeded plates the sterile filter paper discs of 5 mm soaked with 15 µL of lichen extract (200mg/2ml) concentration were placed. The inoculated plates with bacteria were incubated

at 37°C for 24 hrs while fungal plates were incubated at 27°C for 2-3 days.

Streptomycin (10µg/ml) for bacterial and Ketoconazole (15µg/ml) for fungal pathogens were taken as a standard positive control and solvent alone as negative control. All the experiments were carried out in triplicates. Growth was evaluated after 18–24 hrs of incubation in the case of bacterial cultures and similarly, in the case of fungal cultures the plates were observed after 2–3 days. The diameters of the zone of inhibitions were measured in millimeter (including size of paper disc) and the mean and standard deviations are calculated.

RESULTS

Antibacterial activity of extract

Antimicrobial activity was done by using Kirby-Bauer disc diffusion assay with 2-Propanol, Acetone, Petroleum ether extract of *P. andinum* showed their effective results against all the bacterial pathogens. 2-Propanol extracts of Bacterial pathogens showed their maximum zone of inhibition against *Corynebacterium rubrum* (10.3±0.5mm), *Salmonella typhimurium*, (10.0±2.6), *Pseudomonas aeruginosa* (9.6±1.5) and minimum zone of inhibition against *Enterobacter cloacae* (7.3±0.5) were recorded. Acetone extract showed their highest zone of inhibition against *Enterobacter cloacae* (8.3±2.3) and *Streptococcus pyogenes* (8.3 0.5). Lowest zone of inhibition with acetone extract was reported against *Salmonella abony* (4.6±4.0). Petroleum ether extract showed their highest zone of inhibition against *Salmonella abony* (9.3±2.1) and lowest zone of inhibition with petroleum ether extract was reported against *Klebsiella pneumonia* (5.0±4.3). Against the standard drug Streptomycin, bacterial pathogen showed their highest zone of inhibition against *Streptococcus pyogenes* (30.3±1.1) and *Bacillus subtilis* (29.6±1.5). Their minimum zone of inhibition against *Enterobacter cloacae* (24.3±6.6) followed by *Staphylococcus aureus* (23.6±1.1) and *Bacillus cereus* (23.0±0.0). The inhibitory effects of methanol and water extracts on the bacterial pathogens were nil. Inhibition zone of all the bacterial pathogens are provided with their mean and standard deviations in **Fig. 3.**

Antifungal activity of extract

The fungal pathogens *Trichoderma lignorum* (22.66±6.17) and *Aspergillus niger* (21.33±1.20) showed their maximum zone of inhibition with 2-Propanol extract. Minimum zone of inhibition against the fungal pathogens was reported against *Mucor* sp. (12.66±0.66) and *Colletotrichum falcatum* (12.33±1.45). Acetone extract showed their highest zone of inhibition against *Rhizoctonia bataticola* (14.3±1.20) followed by *Aspergillus niger* (13.0±1.52) and *Penicillium chrysogenum* (10.66±0.33) while *Aspergillus flavus* (7.33±0.33), *Fusarium solani* (7.33±0.33) and *Fusarium oxysporum* (5.33 ± 2.72) showed minimum zone of inhibition. The positive control Ketoconazole showed highest zone of inhibition against *Trichoderma lignorum* (20.67±4.61) *Fusarium oxysporum* (15.33±1.52) and *Colletotrichum falcatum* (15.0±1.0) while lowest inhibition zone was reported against *Aspergillus niger* (9.0±2.64), *Mucor* sp. (10.33±1.24) and *Fusarium moniliforme* (11.0±4.0) (Fig. 3A–H and Table 2). The inhibitory effect of methanol, petroleum ether and water extracts on the fungal pathogens were showed negative result. Inhibition zone of all the fungal pathogens are provided with their mean and standard deviations in **Fig. 4.**

DISCUSSION

The present study is mainly focussed on 2-propanol and Petroleum ether extracts of *P. andinum* against different human and plant pathogens. The work has been carried out first time in the study of antimicrobial activity. Earlier work has been proved that 2-propanolic extract of *Roccella montagnei* Bél collected from Horsley hill, Chittoor District can be used for the antimicrobial activities against human pathogenic microorganisms Anjali *et al.* (2014). Sati *et al.* (2011) used *Parmotrema nilgherrense* extracts of Chloroform, Ethanol and Methanol and recorded maximum zone of inhibition against *Bacillus subtilis* and *Escherichia coli* by using 200µL of the extract while in the present study, *P. andinum* extracts inhibited the same bacterial pathogens with a very less quantity (15 µL) of the bioactive compound.

Srivastava *et al.* (2013) has reported that the secondary compounds of extracts of *Usnea longissima*, *Everniastrum cirrhatum*, *Peltigera polydactylon* and *Sulcaria sulcata* in acetone, methanol and ethanol were used against the bacterial species in which *Staphylococcus aureus* and *Bacillus cereus* showed activity with acetone and methanol extracts only against *Usnea longissima*, *Everniastrum cirrhatum* and *Sulcaria sulcata*. The extracts of acetone, methanol and ethanol had no effect against the bacterial species such as *Escherichia coli* and *Salmonella typhimurium*. The bacterial species *Pseudomonas aeruginosa* showed its activity with only the extracts of *Usnea longissima*, *Everniastrum cirrhatum* and no activity were reported with *Peltigera polydactylon* and *Sulcaria sulcata*. The bioactive compound of *P. andinum* showed its effective zone of inhibition against the bacterial species viz. *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa* with 2-Propanol, Acetone and petroleum ether extracts at low concentration.

Table–1. Antibacterial activity of *P. andinum*

S. No.	Bacterial Pathogens	Diameter of Zone of inhibition (mm)			
		Control (S)	2Pol	Acetone	PE
1	*Bacillus cereus*	23.0 ± 0.0	8.3 ± 0.5	8.0 ± 1.0	7.3 ± 0.5
2	*Bacillus subtilis*	29.6 ± 1.5	9.0 ± 1.0	8.0 ±0.0	7.6 ± 0.5
3	*Corynebacterium rubrum*	28.3 ± 1.1	10.3± 0.5	8.0 ± 1.0	8.0 ± 1.0
4	*Enterobacter cloacae*	24.3 ± 6.6	7.3 ± 0.5	8.3 ± 2.3	7.6 ± 0.5
5	*Escherichia coli*	29.0 ± 1.7	9.6 ± 1.1	8.0 ± 1.0	7.3 ±0.5
6	*Klebsiella pneumonia*	26.6 ± 1.5	8.0 ± 1.0	7.3± 0.5	5.0 ± 4.3
7	*Pseudomonas aeruginosa*	27.3 ± 3.0	9.6 ± 1.5	8.0 ± 1.0	7.3 ± 0.5
8	*Salmonella abony*	25.3 ± 4.6	7.3± 0.5	4.6 ± 4.0	9.3 ± 2.1
9	*Salmonella typhimurium*	28.3 ± 0.5	10.0 ± 2.6	8.0 ± 0.0	7.3 ± 0.5
10	*Staphylococcus aureus*	23.6 ± 1.1	9.0 ± 0.0	7.6 ± 0.5	7.0 ± 0.0
11	*Streptococcus pyogenes*	30.3 ±1.1	7.6± 5.7	8.3 ± 0.5	7.0 ± 0.0

S = Streptomycin, 2Pol=2-Propanol, PE = Petroleum ether, ± = mean and standard deviation.

Table–2. Antifungal activity of *P. andinum*

S.No	Fungal pathogens	Diameter of zone of inhibition		
		Control (K)	2pol	Acetone
1	*Aspergillus flavus*	9.33±0.33	15.66±1.15	7.33±0.33
2	*Aspergillus niger*	12.66±1.33	21.33±1.20	13.0±1.52
3	*Colletotrichum falcatum*	16.33±2.72	12.33±1.45	0.0±0.0
4	*Fusarium moniliforme*	11.33±1.85	20.66±0.88	8.33±0.88
5	*Fusarium oxysporum*	18.0±3.21	19.66±2.18	5.33±2.72
6	*Fusarium solani*	11.0±0.57	19.0±1.52	7.33±0.33
7	*Mucor sp.*	12.0±0.57	12.66±0.66	10.33±1.20
8	*Penicillium chrysogenum*	10.66±0.88	20.33±0.66	10.66±0.33
9	*Penicillium notatum*	10.33±0.33	15.0±1.73	8.33±.33
10	*Rhizctonia bataticola*	8.33±1.33	19.33±3.84	14.33±1.20
11	*Trichoderma lignorum*	18.0±3.05	22.66±6.17	0.0±0.0

K = Ketoconazole, 2Pol = 2-Propanol, ± = mean and standard deviation.

Tiwari *et al.* (2011) proved the antifungal activity *of Bulbothrix setschwanensis*, *Evernariastrum nepalense*, *Heterodermia diademata* and *Parmelia thomsonii* with acetone, methanol and chloroform extracts against plant pathogens such as *Aspergillus flavus*, *A. fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *F. solani*, *F. rose-um and Pencillium citrinum* acetone and methanol extracts were effective but the present work paid its effective inhibition against all the fungal pathogens with 2-propanol and acetone extracts whereas it has no effect with the same fungal pathogens in methanol, petroleum ether and water extracts. *P. andinum* showed its effective zone of inhibition against all the eleven bacterial pathogens with petroleum ether extract.

Balaji *et al.* (2007) identified the antimicrobial activity of *Parmotrema praesorediosum* with hexane, diethyl ether, ethyl acetate, acetone, methanol and water extracts against eleven bacterial and one fungal pathogen in

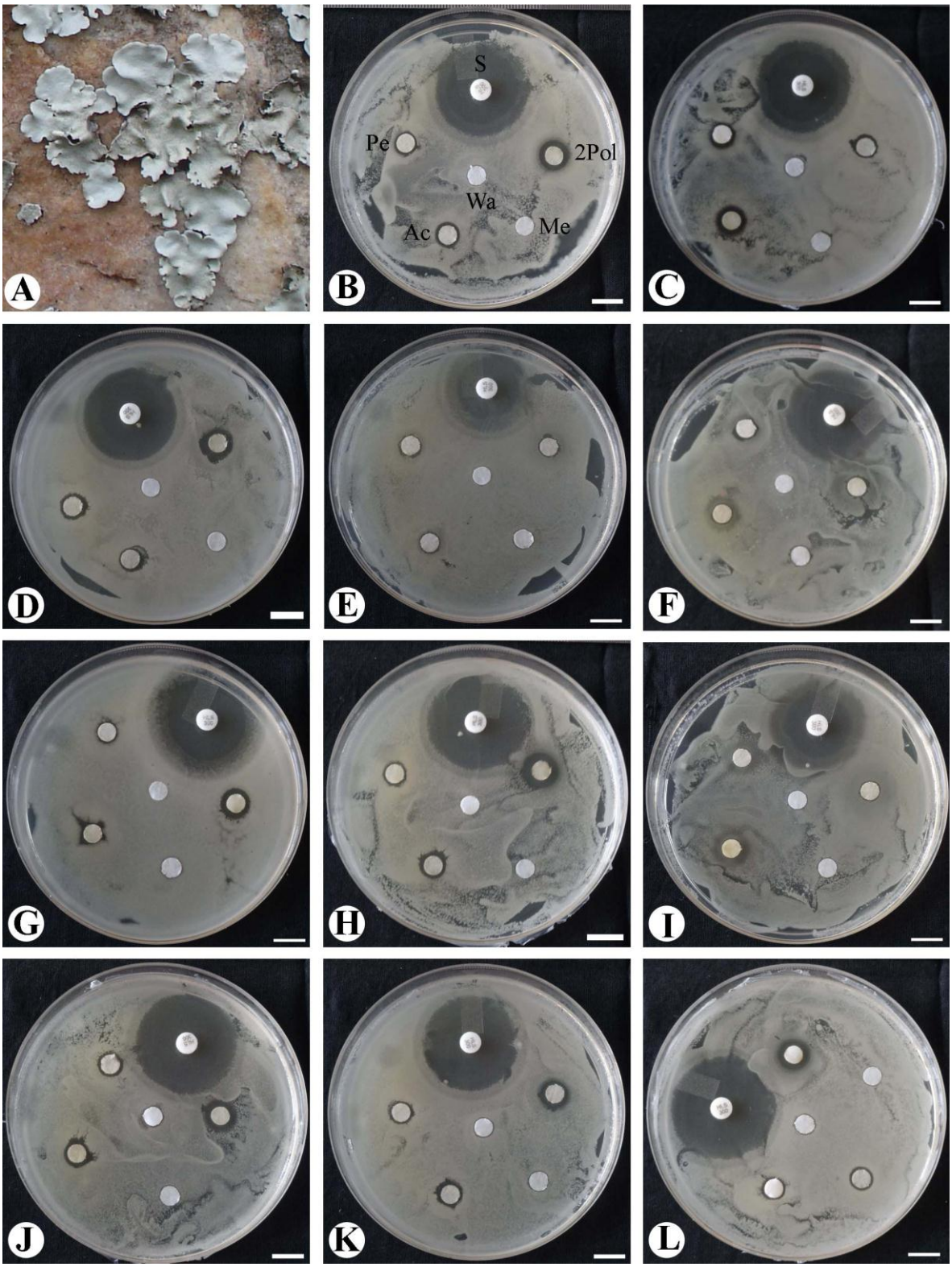

Plate-1: The inhibition zones of tested lichen extract against selected bacterial pathogens and Streptomycin (S), **A.** Habit of *P. andinum*, **B.** *Bacillus cereus*, **C.** *B. subtilis*, **D.** *Corynebacterium rubrum*, **E.** *Enterobacter cloacae*, **F.** *Escherichia coli*, **G.** *Klebsiella pneumonia*, **H.** *Pseudomonas aeruginosa*, **I.** *Salmonella abony*, **J.** *S. typhimurium*, **K.** *Staphylococcus aureus*, **L.** *Streptococcus pyogenes*, **Abbreviations:** 2-Pol=2-propanol, Me= Methanol, Ac= Acetone, Pe= Petroleum ether, Wa= Water, **Scale:** A = 2 cm, B-L =10 mm.

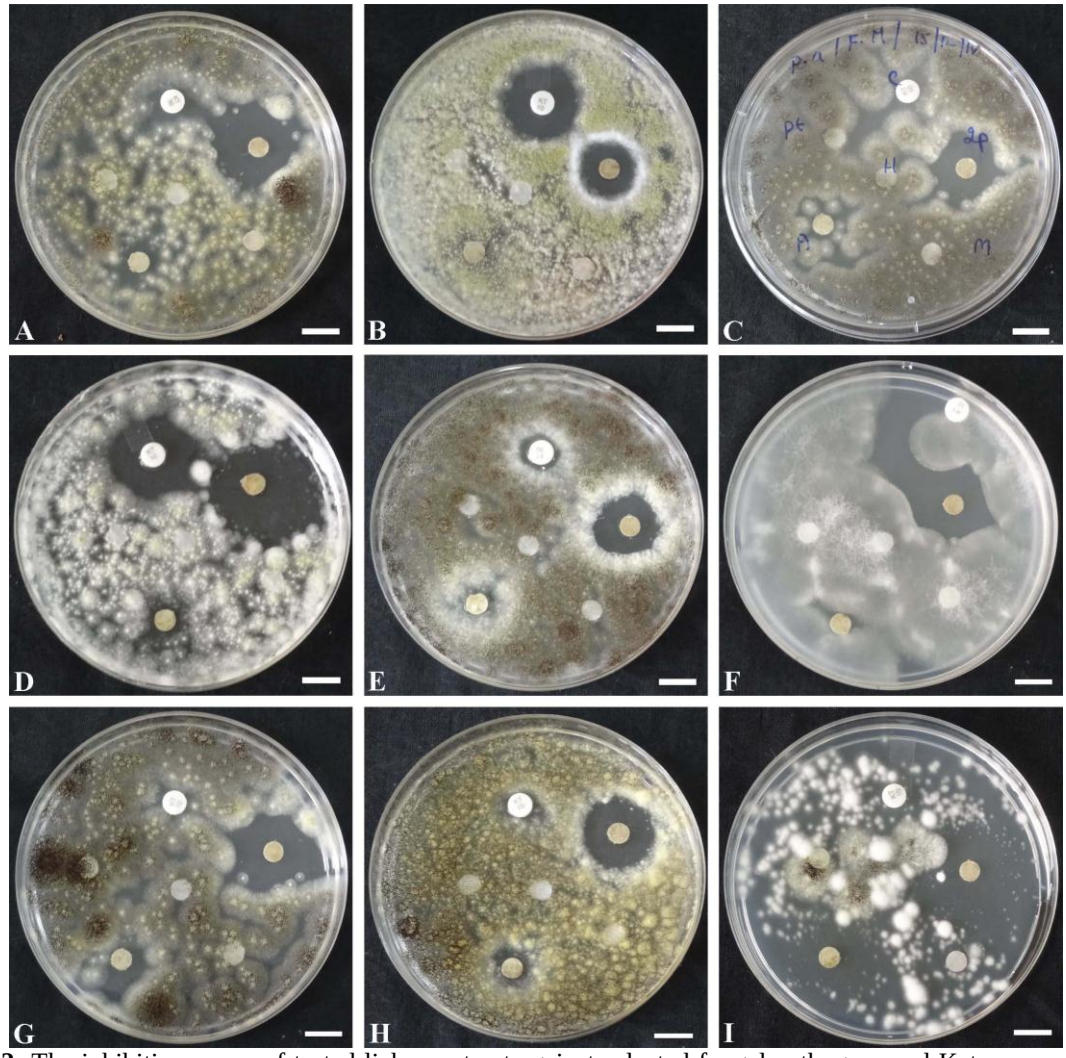

Plate-2: The inhibition zones of tested lichen extract against selected fungal pathogens and Ketoconazole (K), **A.** *A. niger*, **B.** *Colletotrichum falcatum*, **C.** *Fusarium moniliforme*, **D.** *F. oxysporum*, **E.** *F. solani*, **F.** *Mucor* sp. **G.** *Penicillium chrysogenum*, **H.** *P. notatum*, **I**. *Rhizoctonia bataticola*; **Abbreviations;** 2-Pol=2-propanol, Me= Methanol, Ac= Acetone, Pe= Petroleum ether, Wa= Water, **Scale:** A-F = 10 mm.

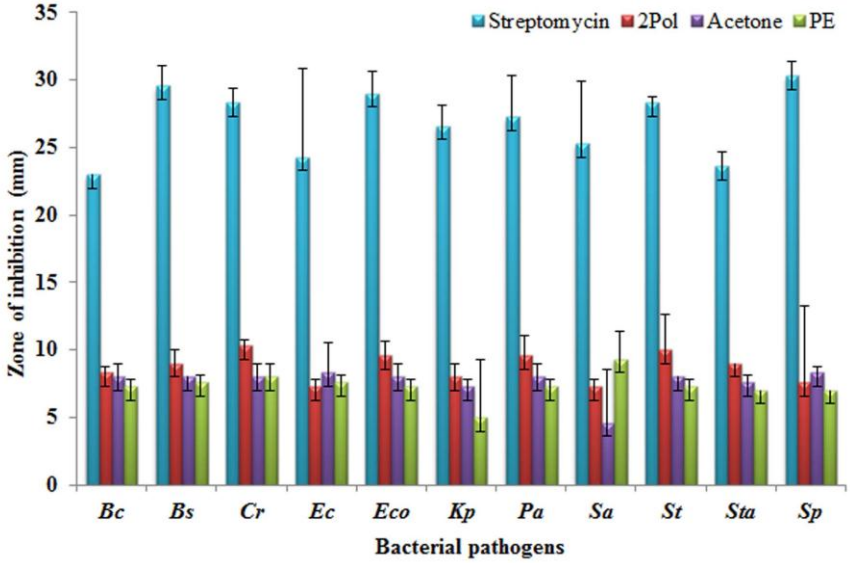

Fig. 1: Comparative statements of 2-propanolic extract of *P. andinum* against selected bacterial pathogens. Streptomycin (control), ***Bc*** = *Bacillus cereus*, ***Bs*** = *B. subtilis*, ***Cr*** = *Corynebacterium rubrum*, ***Ec*** = *Enterobacter cloacae*, ***Eco*** = *Escherichia coli*, ***Kp*** = *Klebsiella pneumonia*, ***Pa*** = *Pseudomonas aeruginosa*, ***Sa*** = *Salmonella abony*, ***St*** = *S. typhimurium*, ***Sta*** = *Staphylococcus aureus*, ***Sp*** = *Streptococcus pyogenes.*

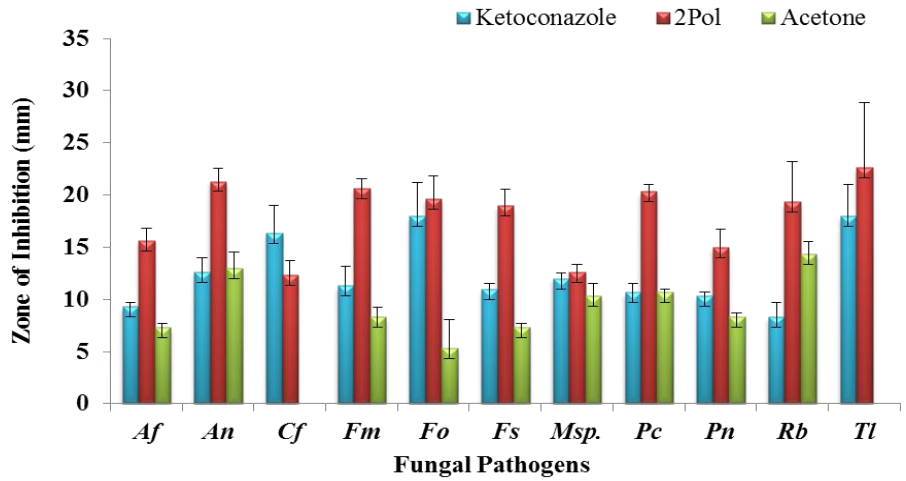

Fig. 2: Comparative statements of 2-propanolic extract of *P. andinum* against selected fungal pathogens. KT = Ketoconazole (control), ***Af*** **=** *Aspergillus flavus*, ***An*** **=** *A. niger*, ***Cf*** = *Colletotrichum falcatum*, ***Fm*** **=** *Fusarium moniliforme*, ***Fo*** **=** *F. oxysporum*, ***M*****sp.** = *Mucor* Sp., ***Pc*** **=** *Penicillium chrysogenum*, ***Pn*** = *Penicillium notatum*, ***Tl*** = *Trichoderma lignorum*.

which the extracts of diethyl ether was active against five bacteria and one fungal pathogen. The secondary compound of *P. andinum* showed its effective zone of inhibition against each eleven bacterial and fungal pathogens with 2-Propanol, acetone and petroleum ether extracts but there was no zone of inhibition with methanol against bacterial and fungal pathogens. Hence, the present study throws much emphasis on antimicrobial activity of the bioactive compound of *P. andinum*. The need for its complete structural analysis of Orsellenic and Lecanoric acids is suggested.

CONCLUSION

Prior to there was no information available on the antimicrobial properties of *P. andinum*, so it paved an enthusiastic attention towards the study about the species. The result obtained from the present study concluded that *P. andinum* has a very extensive antimicrobial activity with the 2-Propanol, acetone and petroleum ether extracts. However, these activities of the extracts are relatively lesser than that of the control used. The study encourages exploring for novel antimicrobial bio-molecules within lichen biodiversity. The lichen *P. andinum* may also yield potential antimicrobial compound in some other organic solvent systems. It is very useful for the pharmacological, tobacco and food industries.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Science and Technology (INSPIRE) and Director and Dr. D.K. Upreti, Chief Scientist, CSIR-National Botanical Research Institute, Lucknow for their kind permission to utilize the infrastructure facilities of Lichenology Laboratory for the identification of lichens. The authors are also thankful to the Forest Official of Andhra Pradesh for their help in conducting the field trips.

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